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3 MOLECULARLY-IMPRINTED MATERIAL MADE BY TEMPLATE-DIRECTED
4 SYNTHESIS
5

6 Background of the Invention

7 1. Field of the Invention

8 The invention relates generally to molecularly-imprinted material and in particular to
9 molecularly-imprinted material made by template-directed synthesis.

10 2. Description of the Related Art

11 Enzymes are commonly exploited for practical uses, including as catalysts in synthetic
12 processes, as detection reagents in chemical and biological sensors, and as catalysts in
13 decontamination of environmental pollutants and other toxic agents. Their usefulness is largely due
14 to their exquisite functional selectivity and regio- and stereospecificity. However, the usefulness of
15 enzymes for practical purposes is limited by their intolerance to harsh conditions, particularly to
16 conditions involving nonaqueous environments, temperature extremes, or the presence of materials
17 that are toxic to the enzyme. In addition, enzymes may have a short shelf-life under ambient
18 conditions and may require refrigerated storage to remain active.

19 Similarly, antibodies are useful for practical purposes such as for detecting or separating
20 specific materials in complex mixtures. As with enzymes, the usefulness of antibodies is due to their

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1 functional selectivity and regio- and stereospecificity. Also, as with enzymes, their usefulness is
2 limited by their intolerance for harsh conditions.

3 As a result of the difficulties in exploiting enzymes and antibodies on a large scale and in
4 harsh environments, efforts have been made to develop enzyme and antibody mimics, that is,
5 materials that can function as enzymes or antibodies, but which have a more durable composition.
6 Specifically, efforts have been made to utilize the principles of molecular recognition to create
7 artificial enzyme active sites or antibody binding sites through molecular imprinting of enzyme
8 transition state analogs or antibody antigens in polymers and inorganic matrices. For example,
9 molecularly printed materials are described in the following patents and publications incorporated
10 herein by reference: U.S. Patent No. 5,110,833 to Mosbach; U.S. Patent No. 5,310,648 to Arnold
11 et al; U.S. Patent No. 5,372,719 to Afeyan et al; U.S. Patent No. 5,453,199 to Afeyan et al; U.S.
12 Patent No. 5,461,175 to Fischer et al; U.S. Patent No. 5,587,273 to Yan et al; U.S. Patent No.
13 5,630,978 to Domb; U.S. Patent No. 5,641,539 to Afeyan et al; U.S. Patent No. 5,728,296 to Hjertén
14 et al; U.S. Patent No. 5,750,065 to Kilbane II; U.S. Patent No. 5,756,717 to Paliwal et al; U.S. Patent
15 No. 5,786,428 to Arnold et al; U.S. Patent No. 5,814,223 to Hjertén et al; U.S. Patent No. 5,821,311
16 to Mosbach et al; U.S. Patent No. 5,858,296 to Domb; U.S. Patent No. 5,872,198 to Mosbach et al.;
17 Mosbach, K. et al, "The Emerging Technique of Molecular imprinting and Its Future Impact on
18 Biotechnology" , Biotechnology, vol 14, February 1996, pp 163 - 170; G. Wulff, "Molecular
19 Imprinting in Cross-Linked Materials with the Aid of Molecular Templates- A Way towards
20 Artificial Antibodies" Angew. Chem. Intl. Ed. Engl., 34, 1812-1832 (1995); P. Hollinger, et al.,

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1 "Mimicking Nature and Beyond" Trends in Biochemistry, 13(1), 79 (1995); Haupt, K., Mosbach,
2 K. Trends Biotech, 16, 468-475 (1997); Davis et al, "Rational Catalyst Design via Imprinted
3 Nanostructured Materials" Chem. Mater. 8 (1996) pp 1820-1839. and Wulff, G. et al, "Enzyme
4 models Based on Molecularly Imprinted Polymers with Strong Esterase Activity" Angew. Chem.
5 Int. Ed. Engl., 36 1962 (1997).

6 During a typical imprinting process, a molecule to be imprinted is combined with a mixture
7 of functionalized and non-functionalized monomers so that the monomers surround the molecule to
8 be imprinted. In the process, functionalized monomers align themselves in a binding relationship to
9 complementary functional groups on the imprint molecule. The monomers are then polymerized,
10 thereby encasing the imprint molecule within the polymer. The imprint molecule is then washed
11 away, and the resulting material contains imprinted binding sites which are the "negative" of the
12 imprint molecule. The complementary binding groups, arising from the functionalized polymer
13 groups incorporated during the imprinting, are specifically positioned to enhance the preferential
14 substrate binding and, if desired, subsequent catalysis.

15 To date, the methods of molecular imprinting described above have achieved only modest
16 success in producing imprinted materials that exhibit selectivity and catalytic activity. The reason
17 for this is that in order to be effective in wide scale use, antibody and enzyme mimics must have
18 binding/active sites that are nearly homogeneous (in specificity and activity), well formed (based on
19 shape and reactivity), and easily accessed by reactant molecules (access is affected by shape, size
20 and polarity of the channels leading to the catalytic site). Site homogeneity and site accessibility are

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1 both equally important. The imprinted sites created by currently known methods are generally not
2 very accessible and are generally not homogeneous, that is, they often have different binding
3 affinities and/or reactivities. These problems arise from the methods used in forming the polymer
4 imprint and providing access to the binding sites. Using the conventional imprinting process, the
5 imprinted sites are completely encased within the polymer. In order to enable access to the sites, the
6 polymer may be ground up, thereby exposing the sites. However, doing so causes the deformation
7 of a large number of the binding sites and irreversibly alters the shape-specificity and the
8 complementary binding of the sites, thereby adversely affecting their selectivity and activity. In an
9 alternative method of enabling access to the imprinted sites, porogens (typically inert solvents) may
10 be incorporated among the polymerizable monomers in the imprinting process. After polymerization,
11 the porogens are washed away, creating pores that allow access to the binding sites. However, as the
12 porogens are removed, some of the structural integrity of the polymer is lost, leading to deformation
13 of the sites. The resultant loss in specificity and activity is similar to that observed as a result of
14 grinding up the polymer.

15 Recently, efforts have been made to improve accessibility by creating imprinted sites on
16 silica or polymer surfaces. In general, this approach involves linking complementary hydrogen-
17 bonding functionalized silanes to the imprint molecule and then creating the molecular recognition
18 site by attaching this "scaffolding" to the surface of a silica or polymer particle. After the imprint
19 molecule is washed away, a binding site with affinity for specific molecules remains on the surface
20 of the particle. This approach is described in the following publications incorporated herein by

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1 reference: Lele B.S, et al "Molecularly Imprinted Polymer Mimics of Chymotrypsin 1. Cooperative
2 Effects and Substrate Specificity" React. Funct. Polym 39(1), 37 - 52 (1999); Lele, B.S.,
3 "Molecularly Imprinted Polymer Mimics of Chymotrypsin 2. Functional Monomers and Hydrolytic
4 Activity" React. Funct. Polym 40(3), 215 - 229 (1999); and Hwang K-O, et al, "Template-Assisted
5 Assembly of Metal Binding Sites on a Silica Surface", Mater. Sci. Eng. C, 3, 137 (1995).

6 This approach has some important limitations: First, the scaffolding process places the
7 imprint molecule on the surface of the particle. Consequently, this procedure imprints only the
8 functionality of the imprint molecule and not the molecule's shape. Additionally, there are limits to
9 how much of the imprint molecule's functionality can be imprinted using this procedure. This is
10 essentially a 2-dimensional form of imprinting in that only those functional groups of the imprint
11 molecule with pre-attached complementary binding groups oriented towards the particle surface
12 would be imprinted. Functional groups with pre-attached complementary binding groups oriented
13 away from the surface would not be tethered to the surface and so would not be imprinted. The fewer
14 functional groups imprinted, the lower the selectivity of the imprinted site for the target molecule,
15 and the binding of the target molecule also will be much weaker. Second, because of the chemistry
16 involved in attaching the imprint molecule-complementary groups complex to a surface, the
17 "scaffolding" procedure is limited to the imprinting of particle or planar surfaces. This procedure is
18 not useful for imprinting porous materials due to difficulties in introducing the reactants into the
19 pores. Even if the attachment to the surfaces of the pores could be achieved, this imprinting would
20 necessarily restrict the flow of any target molecules through the pores, thereby creating the

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1 accessibility problems this approach was designed to alleviate.

2 In a separate field of technology, methods have been developed for making particles and
3 porous materials by template-directed synthesis. In these methods, surfactants are used to create
4 molecular microstructures such as micelles or reverse micelles in a solvent medium and then
5 inorganic or organic monomers are polymerized around the molecular microstructures at the
6 surfactant-solvent interface. When the surfactant is removed, the remaining material has a size and
7 shape complementary to the size and shape of the molecular microstructures. By controlling
8 variables such as surfactant selection and concentration, a variety of different microstructure shapes
9 such as micellar, cubic, tetragonal, lamellar, tubular and reverse micellar can be formed and,
10 consequently, monodisperse particles of a variety of different sizes and porous materials with a
11 variety of different shapes of pores and channels can be created. Methods of making porous material
12 are described, for example, in the following patents and publications incorporated herein by
13 reference: U.S. Patent No. 5,250,282 to Kresge et al; U.S. Patent No. 5,304,363 to Beck et al; U.S.
14 Patent No. 5,321,102 to Loy et al; U.S. Patent No. 5,538,710 to Guo et al; U.S. Patent No. 5,622,684
15 to Pennavaia et al; U.S. Patent No. 5,750,085 to Yamada et al; U.S. Patent No. 5,795,559 to
16 Pinnavaia et al; U.S. Patent No. 5,786,294 to Sachtler et al; and U.S. Patent No. 5,858,457 to Brinker
17 et al; J. C. Vartuli, et al, "Effect of Surfactant/Silica Molar ratios on the Formation of Mesoporous
18 Molecular Sieves: Inorganic Mimicry of Surfactant Liquid-Crystal Phases and Mechanistic
19 Implications" Chemistry of Materials, 6, 2317 2326 (1994); C.A. Morris, et al "Silica Sol as a
20 Nanoglue: Flexible Synthesis of Composite Aerogels" Science, 284, 622-624 (1999); B.T. Holland

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1 et al, "Synthesis of Highly Ordered, Three-Dimensional, Macroporous Structures of Amorphous or
2 Crystalline Inorganic Oxides, Phosphates and Hybrid Composites" Chem Mater 11, 795-805 (1999);
3 and M. Antonietti, et al, "Synthesis of Mesoporous Silica with Large Pores and Bimodal Pore Size
4 Distribution by Templating of Polymer Latices" Advanced Materials 10, 154-159 (1998). These
5 materials, while being able to distinguish molecules on the basis of size, typically lack the
6 specificity and activity of enzymes and antibodies. Methods for making monodisperse silica particles
7 by hydrolyzing alkoxysilanes in a surfactant-stabilized water-in-oil microemulsion containing
8 ammonia are described, for example, in the following patents and publications incorporated herein
9 by reference: U.S. Patent No. 5,209,998 to Kavassalis et al; W. Stober et al, "Controlled Growth of
10 Monodisperse Silica Spheres in the Micron Size Range" J. Colloid Interface Sci., 26, 62 (1968);
11 Lindberg et al, "Preparation of Silica Particles Utilizing the Sol-Gel and the Emulsion-Gel
12 Processes" Colloids and Surfaces A 99, 79 (1995); P. Espiard et al, "A Novel Technique for
13 Preparing Organophilic Silica by Water-In Oil Microemulsions" Polymer Bulletin, vol. 24, 173
14 (1990); H. Yamauchi et al, "Surface Characterization of Ultramicro Spherical Particles of Silica
15 Prepared by W/O Microemulsion Method", Colloids and Surfaces, Vol 37, 71 - 80 (1989);
16 Markowitz et al, "Surface Acidity and Basicity of Functionalized Silica Particles" Colloids and
17 Surfaces A: Physicochem Eng. Aspects 150, 85 - 94 (1999). The formation of silica gel-coated metal
18 and semiconductor nanoclusters is described in U.S. Patent No. 5,814,370 to Martino et al. As
19 described below, unfunctionalized silica particles have little or no catalytic activity; catalytic activity
20 is increased with functionalized silica particles, but not to the level achieved with imprinted

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1 materials.

2 **Summary of the Invention**

3 Accordingly, it is an object of the present invention to provide an imprinted material and a
4 method of making an imprinted material wherein the imprinted sites are easily accessible to target
5 molecules.

6 It is a further object of the present invention to provide an imprinted material and a method
7 of making an imprinted material wherein the imprinted sites are nearly homogeneous in activity and
8 specificity.

9 These and other objects are achieved by a method for making a molecularly imprinted solid
10 structure by the steps of: providing a surfactant compound having a headgroup, wherein the
11 headgroup comprises a group to be molecularly imprinted; combining the surfactant compound with
12 a solvent to form a mixture and maintaining the mixture from the previous step so that molecules
13 of the surfactant self-assemble to form at least one supramolecular structure having exposed imprint
14 groups; combining the mixture with at least one reactive monomer so that the supramolecular
15 structure serves as a template for the organizing of molecules of the reactive monomer surrounding
16 or in contact with the supramolecular structure, including surrounding the imprint groups;
17 maintaining the mixture so that the reactive monomers react with each other to form a solid structure
18 having a shape complementary to the shape of the supramolecular structure, including the shape of
19 the exposed imprint groups; and removing the supramolecular structure from the solid structure. The
20 resulting structure is a porous material or particle having molecularly imprinted sites. Because the

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imprinting is accomplished into the material's surface, the imprinted sites are easily accessible to target molecules. And because no drastic processing steps such as grinding are required to achieve accessibility, the imprinted sites tend to be homogeneous in specificity and reactivity.

Brief Description of the Drawings

Figure 1 shows the kinetics of the hydrolysis of DL-BAPNA catalyzed by non-imprinted silica particles (●) and silica particles surface-imprinted with 10% (○), 20% (▼), and 40% (▽) N- α -decyl-L-phenylalanine-2-aminopyridine. All particles contain 5 wt % (total silica) of a mixture of PEDA, IPTS, and CTES.

Figure 2 shows the kinetics of the hydrolysis of various substrates by silica particles surface-imprinted with N- α -decyl-L-phenylalanine-2-aminopyridine. All particles contain 5 wt % (total silica) of a mixture of PEDA, IPTS, and CTES.

Detailed Description of the Preferred Embodiments

The present invention provides methods for making molecularly imprinted particles and porous materials having accessible and homogeneous imprinted sites.

As used herein, the term "molecularly imprinted" material refers to material that has been polymerized around molecules of an imprint compound in such a way that when the imprint group is removed from the material, cavities or "imprinted sites" remain in the material that are negative

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1 images of the imprint compound. When the molecularly imprinted material is subsequently exposed
2 to a solution containing the imprint compound, the imprinted sites selectively bind the imprint
3 compound. This selective binding allows the material to be used as an artificial antibody or enzyme.
4 For even greater selectivity of the molecularly imprinted material, functional monomers with
5 complementary binding affinity to functional groups on the imprint compound can be incorporated
6 into the material.

7 To make a molecularly imprinted material according to the present invention, an imprint
8 group is coupled to, or forms the headgroup of, a surfactant. The surfactant is then maintained in a
9 mixture under conditions in which the molecules of the surfactant form at least one supramolecular
10 structure and wherein the imprint groups are exposed on the surface of the supramolecular structure.
11 The solution is combined with reactive monomers (which can include functionalized monomers)
12 under sufficient conditions so that the reactive monomers surround the supramolecular structure,
13 including the exposed imprint groups. The reactive monomers are polymerized to form a solid
14 structure that has the negative shape of the supramolecular structure. That is, it has a surface
15 topography that is complementary to the surface topography of the supramolecular structure. The
16 supramolecular structure is then removed, and what is left behind is a solid structure that has pores,
17 channels, or other types of voids corresponding to the shape of the supramolecular structure. Because
18 the imprint groups were exposed on the surface of the supramolecular structure when the solid
19 structure was formed, the surfaces or walls of the voids include imprint sites, which are indentations
20 in the surface having a shape complementary to the shape of the imprint group. These imprint sites,

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1 because they are created on the surfaces or walls of voids, are extremely accessible, much more so
2 than when the imprint group alone is used to create a molecularly imprinted material.

3 By this method, a porous structure having pores or channels and having molecularly
4 imprinted indentations or cavities in the walls or surfaces of the pores or channels may be created.

5 Typically, the pores or channels may be in the size range of about $0.01\mu\text{m}$ to about $0.5\mu\text{m}$ or greater

6 Alternatively, molecularly imprinted particles may be formed by combining an imprint
7 group-coupled surfactant as described above with an organic solvent and water to form a water-in-oil
8 microemulsion. In the microemulsion, molecules of the surfactant arrange themselves so that the
9 imprint groups are in the water phase and the hydrophobic tails are in the organic phase. When a
10 water-soluble reactive monomer is added and caused to polymerize in the water phase, the particles
11 that are formed have indentations in their surfaces that are the mirror image of the imprint groups.
12 If functionalized reactive monomers are also added to the microemulsion, the imprinted sites of the
13 particles will include functional groups in a spaced-apart arrangement corresponding to the location
14 of complementary functional groups on the imprint group. By this method, imprinted particles of
15 a variety of sizes can be created. For example, imprinted particles having a narrow size distribution
16 and having an average size of between about 50 and about 1200 nm can be created.

17 The imprint group in the present invention can be any chemical entity for which it would be
18 useful to have imprinted sites. For example, if the imprinted material is to be used as an artificial
19 antibody, the imprint group can be the compound or a portion of the compound sought to be bound
20 by the antibody. If the imprinted material is to be used as an enzyme, the imprint group can be a

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1 transition state analog (TSA), that is, an analog of the transition state that is formed during the
2 enzymatic conversion of a substrate to a product. The imprint group may also be an inhibitor (a
3 compound that inhibits enzyme activity), a substrate or a substrate analog of the enzyme.

4 The surfactant in the present invention can be any compound such as, for example, an
5 amphiphilic compound, that is capable of self-assembling in solution to form one or more
6 supramolecular structures or any monomeric or polymeric surfactant that can form lamellar and/or
7 non-lamellar phases. The imprint group is coupled to the surfactant by any sufficient means so that
8 when molecules of the surfactant form one or more supramolecular structures, the imprint groups
9 are exposed on the surface of the supramolecular structures. Preferably, the surfactant is an
10 amphiphilic compound having a hydrophilic headgroup and a hydrophobic tail, and the imprint
11 group makes up, or forms part of, the headgroup of the compound. (Such a surfactant can be created,
12 for example, by acylating a hydrophilic imprint compound so that the combination of a hydrophilic
13 headgroup and a hydrophobic acyl tail gives the compound the amphiphilic properties of a
14 surfactant.) Due to thermodynamic driving forces, molecules of an amphiphilic surfactant self-
15 assemble in an aqueous medium to minimize the exposure of their hydrophobic portions to the
16 medium, while maximizing the exposure of the hydrophilic headgroup. Consequently, if a surfactant
17 has an imprint group as a headgroup, in whole or in part, the imprint group will be exposed to the
18 surfaces of the self-assembled structure and imprinted sites will be formed when these surfaces are
19 subsequently mineralized or when a mirror-image polymer is created.

20 Alternatively, the imprint group could be attached to the end of a hydrophobic portion of a

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1 surfactant. In this embodiment, a non-aqueous solvent or a solvent combination having an oil phase
2 would be selected so that when a supramolecular structure is formed in a solvent, the imprint group
3 would exposed be to the non-aqueous or oil phase. The organic or inorganic reactive monomers
4 would be selected so that they polymerize in a non-aqueous environment and imprinting would occur
5 at the surface of a polymer formed in the oil phase.

6 The imprint group-coupled surfactant is combined with a solvent and the solution is
7 maintained under conditions of temperature, time, pH and surfactant concentration so that molecules
8 of the surfactant self-assemble into supramolecular structures. The conditions of temperature, time,
9 pH and surfactant concentration that are sufficient depends on the particular surfactant and how it
10 behaves in the particular solvent. The sufficient conditions can be readily determined by
11 experimentation based on what is known about the behavior of non-coupled surfactants. Preferably,
12 the surfactant is selected and paired with a particular solvent wherein it is known that supramolecular
13 structures will form easily.

14 The solution may also contain at least one additional surfactant, that is, a surfactant that does
15 not have an imprint group associated with it. The additional surfactant is selected so that it also self-
16 assembles with the imprint group-coupled surfactant and becomes incorporated into the
17 supramolecular structure. An additional surfactant would be used, for example, in instances where
18 the imprint group is a relatively large molecule compared to the surfactant and wherein it would be
19 desirable to spread out the exposed imprint groups on the surface of the supramolecular structure.

20 The term "supramolecular structure" includes any liquid crystal structure formed by the

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1 arrangement of surfactant molecules in a solvent. Examples of structures include micelles, vesicles,
2 bicontinuous cubic phase, hexagonal phase, tubules or reverse micelles. The size and type of
3 structures that are formed depends on variables such as the temperature, pH, the concentration of
4 the imprint group-surfactant, the identity and concentration of any additional surfactant, the identity
5 of the solvent, the identity and concentration of the reactive monomer, etc. Because the purpose of
6 the supramolecular structure is to provide accessibility for imprinted sites, it will often not be critical
7 which particular liquid crystal structure is formed, as long as a supramolecular structure is formed
8 that can serve as a template and that can hold the imprint groups in an exposed position on the
9 surface of the supramolecular structure. Likewise, in the formation of imprinted particles in a water-
10 in-oil microemulsion, the size of the particles that can be formed is affected by variables such as the
11 temperature, pH, the concentration of the imprint group-surfactant, the identity and concentration
12 of any additional surfactant, the identity of the solvent, the identity and concentration of the reactive
13 monomer, etc.

14 The mixture of the imprint group-coupled surfactant and solvent is combined with reactive
15 monomers. The reactive monomers may be added either before or after the supramolecular structures
16 form. The mixture is maintained under sufficient conditions of time, temperature and monomer
17 concentration so that the monomers surround the supramolecular structure and so that the
18 supramolecular structure acts as a template to direct the organizing and positioning of the monomers
19 around the structure. The sufficient conditions depend on the particular monomers used and are
20 either well known for specific monomers or can be readily determined by experimentation. Because

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1 the imprint groups are exposed on the surface of the supramolecular structure, the reactive monomers
2 surround the imprint groups as well. The addition of the inorganic monomers may induce changes
3 in the supramolecular structure from the micellar surfactant phase to a mesoporous surfactant phase
4 (cubic or hexagonal) during the polymerization or mineralization process, but this would not affect
5 the creation of imprinted sites. In this manner, mesoporous metal oxide materials can be formed in
6 water using an acid or base catalyst. Cationic surfactants, such as cetyltrimethylammonium bromide
7 (CTAB), or nonionic surfactants, such as BRIJ® type (a class of polyoxyethylene alkyl ethers sold
8 by ICI Americas Inc., Wilmington, Del.) or amphiphilic block co-polymers, can be used to form the
9 liquid-crystalline phases (LC) that transform to cubic or hexagonal phases upon complexation with
10 the monomeric inorganic precursor. Subsequent hydrolysis and condensaton of this monomer,
11 followed by removal of the surfactant results in formation of the mesoporous metal oxide.

12 The reactive monomers can be molecules any compound or compounds that are capable of
13 surrounding the supramolecular structure and that can be reacted with each other or polymerized to
14 form a solid or shape-retaining structure with a shape that is complementary to the shape of the
15 supramolecular structure and the exposed imprint groups. The monomers are selected according to
16 the desired end product. For example, to make an inorganic oxide or metal oxide molecularly
17 imprinted material, the monomers may be inorganic oxide or metal oxide precursors. As a more
18 specific example, to make a molecularly imprinted material of silica, the reactive monomers may
19 be alkoxysilanes, particularly tetraalkoxysilanes such as tetraethoxysilane (TEOS),
20 tetramethoxysilane (TMOS), tetrabutoxysilane (TBOS), etc. To make inorganic oxides containing

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1 germanium, titanium or aluminum, reactive monomers include, but are not limited to,
2 tetramethoxygermane, tetraisoprpoxygermane, tetraethoxygermane, tetrabutoxygermane, aluminum
3 n-butoxide, aluminum isopropoxide, titanium ethoxide, titanium diisopropoxide (bis-2,4-
4 pentanedionate), titanium methyl phenoxide, vanadium triisopropoxide oxide, vanadium tri-n-
5 propoxide, zirconium n-butoxide, zirconium n-propoxide, etc. Mixed inorganic or metal oxides may
6 be made by combining inorganic precursors. To make an organic molecularly imprinted material,
7 the reactive monomers may be polymerizable organic monomers including, but not limited to,
8 acrylate, methyl methacrylate, olefins, pyrrole, saccharides, silanes, styrene, isocyanates, vinyl
9 acetate, vinyl chloride, etc. Mixed materials may be made by combining inorganic and organic
10 reactive monomers.

11 The reactive monomers may include functionalized monomers, that is, monomers that have
12 functional moieties with complementary binding affinity for functional moieties on the imprint
13 group by, for example, hydrogen-bonding, electrostatic interactions or other van der Waals
14 interactions. Examples of functional moieties include, but are not limited to, amine (primary,
15 secondary, tertiary, quaternary), hydroxyl, carboxylate, sulfhydryl, amino acids, metal chelates such
16 as iminodiacetic acid-metal ions (divalent or trivalent metal ions), ureido, urea, thiourea, amidine,
17 methyl, phenyl, fluorocarbons, nucleotides, phosphonucleotides, phosphates, saccharides, multiple
18 combinations of any of the these (ie. dicarboxylates, diamines, etc), and multiple combinations of
19 different functional groups. As the reactive monomers surround the supramolecular structure, the
20 functionalized monomers will tend to become spatially distributed and oriented around the imprint

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1 groups so that they form binding complexes with the corresponding functional moieties on the
2 imprint group. Then, as the reactive monomers are polymerized, the functionalized monomers are
3 locked in place. In the formation of a silica imprinted structure using a tetraalkoxysilane such as
4 tetraethoxysilane, examples of functionalized monomers that can be used include 3-
5 (Aminoethylaminomethyl)-phenyltrimethoxysilane (PEDA), carboxyethylsilanetriol (CTES), and
6 N-(3-triethoxysilylpropyl)-4,5-dihydroimidazole (IPTS), 3-aminoalkyltriethoxysilane, N-(2-
7 aminoethyl)-3-aminopropyltrimethoxysilane, decyltriethoxysilane, hydroxymethyltriethoxysilane,
8 isobutylmethyldimethoxysilane, 3-mercaptopropyltriethoxysilane, pentafluorophenylpropyl-
9 trimethoxysilane, phenyltrimethoxysilane, N-trimethoxysilylpropyl-N,N,N-trimethylammonium
10 chloride, ureidopropyltriethoxysilane, and 3-trihydroxysilyl-propylmethylphosphonate, sodium salt.

11 The sufficient conditions under which the monomers react varies depending on the monomer
12 and is either well known for a particular monomer or can be readily determined by experimentation.
13 Inorganic monomers generally polymerize in water in the presence of a catalyst. For example,
14 tetraethoxysilanes and similar monomers hydrolyze in water at room temperature in the presence of
15 an ethanol/ammonia catalyst. Organic monomers polymerize in the presence or absence of a catalyst
16 at varying temperatures, solvent mixtures, pressure, exposure to UV irradiation etc., depending on
17 the particular monomer.

18 After the reactive monomers are reacted to form molecularly imprinted particles or a
19 molecularly imprinted porous structure, the molecules of the surfactant are extracted and removed.
20 This can be done by any means such as, for example, washing the particles or the structure.

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Having described the invention, the following examples are given to illustrate specific applications of the invention including the best mode now known to perform the invention. These specific examples are not intended to limit the scope of the invention described in this application.

EXAMPLES

Molecular imprinting and template directed synthesis were used to create catalytic silica particles that have catalytic activity as esterase mimics. Specifically, L-phenylalanine-2-aminopyridine (a chymotrypsin transition state analog (TSA)) and 3-aminophenyl-boronic acid (a chymotrypsin inhibitor) were acylated to form amphiphilic imprint molecules, which were then mixed with the nonionic surfactant polyoxyethylene(5) nonylphenyl ether (NP-5). These surfactant mixtures were used to form microemulsions that were then utilized to synthesize surface imprinted silica particles from tetraethoxysilane (TEOS) and amine, carboxylate, and dihydroimidazole functionalized silanes. The effects of the surfactant TSA structure, surfactant TSA/NP-5 ratios, and functionalized silane/TEOS ratios on the catalytic activity of the particles is described. In addition, the specificity of these particles is discussed.

Materials and Equipment

3-(Aminoethylaminomethyl)-phenyltrimethoxysilane (PEDA), carboxyethylsilanetriol (CTES), and N-(3-triethoxysilylpropyl)-4,5-dihydroimidazole (IPTS) were purchased from Gelest (Tullytown, PA); Tetraethoxysilane (TEOS), Igepal® CO-520 (polyoxyethylene(5) nonylphenyl ether) (NP-5), 3-aminophenylboronic acid, 2-aminopyridine, dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (TFA), ethanol, cyclohexane, acetonitrile, and dimethyl sulfoxide (DMSO) were

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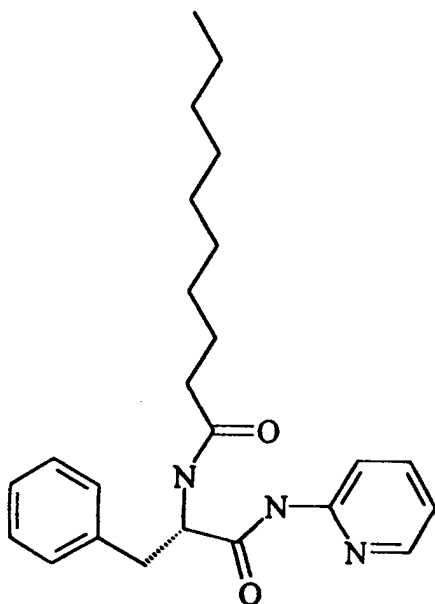
1 purchased from Aldrich Chemical Co. (Milwaukee, WI); N- α -t-BOC-L-phenylalanine was
2 purchased from Calbiochem-Novabiochem Corp. (San Diego, CA); methanol and glacial acetic acid
3 were purchased from Fisher Scientific (Pittsburgh, PA); all chemicals were used as received.
4 Saturated solutions of ammonia in ethanol were prepared by passing ammonia gas into denatured
5 ethanol at 20 °C for 5-6 hours. Electron microscopy was performed with a Zeiss EM-10 transmission
6 electron microscope operated at 60 kV. UV/Vis spectroscopy was performed with a Beckman DU-
7 650 spectrophotometer.

8 **Synthesis of imprint group-coupled surfactants**

9 The following describes the synthesis of two compounds, N- α -decyl-L-phenylalanine-2-
10 aminopyridine and 3-octylamidophenylboronic acid, that were used to create molecularly imprinted
11 polymers by template-directed synthesis. Each of these compounds is a surfactant that comprises a
12 headgroup and an acyl tail, the headgroup being the portion of the compound that is to serve as the
13 imprint group.

14 **Synthesis of N- α -decyl-L-phenylalanine-2-aminopyridine** (a surfactant - α -chymotrypsin
15 transition state analog). This compound has the following structure:

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2 The acylated phenylalanine anilide imprint molecule was synthesized in three steps. (1) N- α -
3 t-BOC-L-phenylalanine was coupled with 2-aminopyridine using DCC to give N- α -t-BOC-L-
4 phenylalanine-2-aminopyridine. (2) The α -amine was then deprotected with TFA to give N- α -L-
5 phenylalanine-2-aminopyridine. (3) To create a compound having the properties of a surfactant, the
6 free amine was then acylated with decanoic acid using DCC to give N- α -decyl-L-phenylalanine-2-
7 aminopyridine.

8 (1) Synthesis of N- α -t-BOC-L-phenylalanine-2-aminopyridine: DCC (4.4 g, 21.1 mmol)
9 was added to a solution of N- α -t-BOC-L-phenylalanine (10 g, 37.7 mmol) in 200 mL THF at 0 °C.
10 The mixture was stirred for 30 minutes and then filtered. 2-Aminopyridine (4.5 g, 41.5 mmol) was
11 added to the filtrate in a round bottom flask and the mixture was stirred overnight. The reaction

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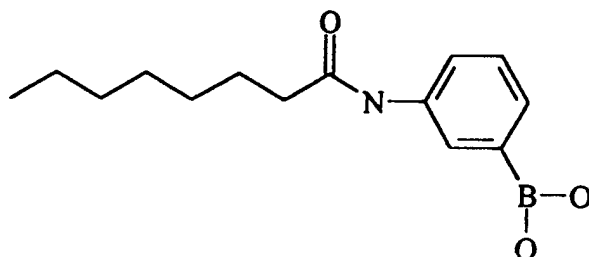
1 mixture was filtered and the solvent was evaporated under reduced pressure until approximately 20
2 mL of THF remained. Petroleum ether was added and the product was precipitated from solution
3 overnight. The semi-solid precipitate collected by filtration was purified by silica gel column
4 chromatography (95:4:1 CHCl₃:CH₃OH:H₂O, v/v/v) to give 8 g (60% yield) of the desired amide.

5 (2) Synthesis of N- α -L-phenylalanine-2-aminopyridine: - α -t-BOC-L-phenylalanine-2-
6 amino-pyridine (2 g, 5.8 mmol) was dissolved in 10 mL 1:1 TFA:CH₂Cl₂ (v/v) and stirred vigorously
7 for 10 min. The TFA:CH₂Cl₂ solution was then evaporated under a stream of N₂. The residue was
8 dissolved in 1 mL CHCl₃ and purified by silica gel column chromatography (80:18:2
9 CHCl₃:CH₃OH:H₂O, v/v/v) to give 1.4 g of N- α -L-phenylalanine-2-aminopyridine in quantitative
10 yield.

11 (3) Synthesis of N- α -decyl-L-phenylalanine-2-aminopyridine: DCC (1.0 g, 4.85 mmol)
12 was added to a solution of decanoic acid (1.50 g, 8.7 mmol) in 10 mL of chloroform under nitrogen
13 atmosphere. A white suspension was formed after stirring for 3 hours at room temperature. The
14 solid was removed by filtration. To the chloroform solution, 10 mL of a THF solution of N- α -L-
15 phenylalanine-2-aminopyridine (1.0 g, 4.1 mmol) was added. After stirring at room temperature for
16 12 hours, a white suspension was formed. The solvents were removed under reduced pressure and
17 the resulting solid was purified first by silica gel column chromatography with a mixture of
18 chloroform/methanol=95:5(v/v) as the eluent, then by crystallization in a mixture of hexanes and
19 toluene to give N- α -decyl-L-phenylalanine-2-aminopyridine as a white crystalline solid (1.1 g, yield:
20 67%).

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Synthesis of 3-Octylamidophenylboronic acid (a surfactant - α -chymotrypsin inhibitor). This compound has the following structure:



Octanoyl chloride (4.6 g, 28.3 mmol) was added slowly to a solution of 3-aminophenylboronic acid (5.0 g, (26.9 mmol) in DMSO (50 mL) containing pyridine (2.3 mL, 28.3 mmol). After addition was complete, the reaction mixture was stirred overnight. The reaction mixture was diluted with 500 mL water and extracted with chloroform. The organic fractions were collected and dried over MgSO_4 , filtered, and then, the solvent was evaporated under reduced pressure to give the crude product as an oil. The oil was redissolved in a small amount of chloroform and passed through a cation exchange column (Bio-Rad AG50W-X8 resin, 20 - 50 mesh, hydrogen form) to remove any pyridine bound to the boronic acid. The crude product was then purified by silica gel column chromatography (98:2 CHCl_3 : CH_3OH , v/v) to give 2.5 g, (35.6 % yield) of the desired product.

Synthesis of particles

The following particles were synthesized:

Silica particles without modification (comparative example)

Silica particles that did not contain functionalized silanes were prepared by stirring a

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1 saturated solution of ammonia in ethanol with cyclohexane, the surfactant Igepal® CO-520
2 (polyoxyethylene(5) nonylphenyl ether) (NP-5), and water for 30 minutes at room temperature and
3 then adding tetraethoxysilane (TEOS). Stirring continued overnight. The volume of the reaction
4 mixture was reduced by vacuum evaporation and the particles were separated from the remaining
5 reaction mixture by centrifugation, washed three times with a wash solution consisting of 4 parts
6 methanol, one part glacial acetic acid, and one part water, followed by washing three times with
7 acetonitrile. The particles were then air dried overnight. Unstained, unwashed particles on copper
8 grids were observed by electron microscopy to determine particle size.

9 **Silica particles with surface amine, dihydroimidazole, and carboxylate groups (Comparative**
10 **example)**

11 Silica particles with surface amine, dihydroimidazole, and carboxylate groups were prepared
12 by including measured amounts of 3-(aminoethylaminomethyl)-phenyltrimethoxysilane (PEDA),
13 carboxyethylsilanetriol (CTES), and N-(3-triethoxysilylpropyl)-4,5-dihydroimidazole (IPTS) to the
14 saturated solution of ammonia/ethanol, cyclohexane, Igepal® CO-520 (polyoxyethylene(5)
15 nonylphenyl ether) (NP-5), and water described above, before stirring and adding TEOS. The
16 particles were separated and dried as described above.

17 **Imprinted silica particles made without functionalized silanes**

18 Imprinted silica particles were prepared by mixing NP-5 with an imprint group-coupled
19 surfactant and solubilizing in ethanol/cyclohexane and then adding the mixture to the
20 ammonia/ethanol mixture. TEOS was then added and particles were formed, separated and dried

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in the same manner as described above.

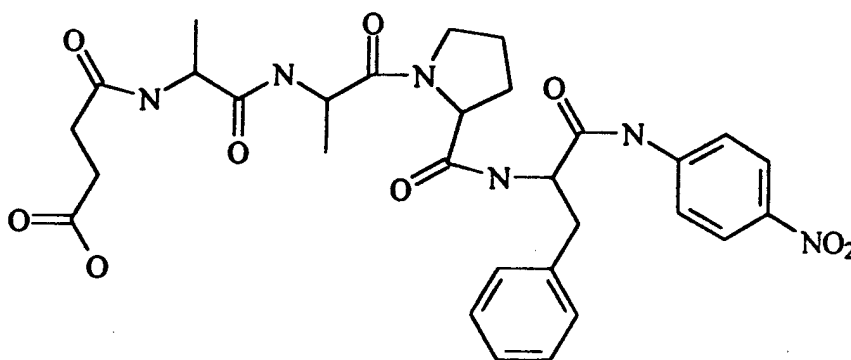
Imprinted silica particles made with functionalized silanes

Imprinted silica particles made with functionalized silanes were prepared by mixing NP-5 with an imprint group-coupled surfactant and solubilizing in ethanol/cyclohexane and then adding the mixture to the ammonia/ethanol mixture. 3-(Aminoethylaminomethyl)-phenyltrimethoxysilane (PEDA), carboxyethylsilanetriol (CTES), and N-(3-triethoxysilylpropyl)-4,5-dihydroimidazole (IPTS) were added before stirring and TEOS was added. The particles were formed, separated and dried in the same manner as described above.

Assay for hydrolytic activity.

Hydrolytic activity of the silica particles was determined by observing the hydrolysis of succinyl-Ala-Ala-Pro-Phe-*p*-nitrophenyl ester (Suc-AAPF-PNP), a chymotrypsin substrate, and benzoyl-DL-arginine-*p*-nitrophenyl ester (DL-BAPNA), a standard chromogenic substrate of trypsin.

Suc-AAPF-PNP has the following structure:

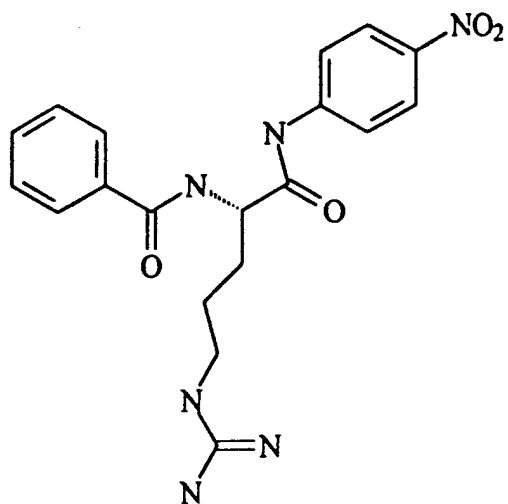


L-BAPNA has the following structure:

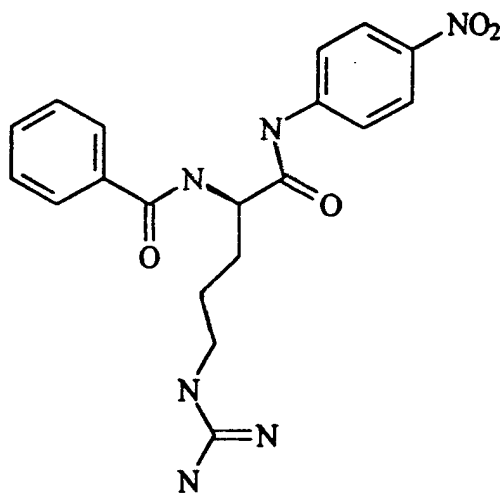
Docket No.: N.C. 79,430

PATENT APPLICATION

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2
3 D-BAPNA has the following structure:



6
7 Substrate stock solutions were prepared by dissolving ~50 mg BAPNA or suc-AAPF-PNP
8 in DMSO; these stock solutions were then diluted with DMSO and 0.1 M TRIS/HCl buffer (pH 7.4)
9 to provide substrate solutions of various concentrations (final concentration of DMSO in each

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1 substrate solution was 15 % v/v). A measured quantity of particles, 30-100 mg, were placed in a
2 microcentrifuge tube, 1.3 ml of substrate solution was added, and the mixture was bath-sonicated
3 to completely disperse the particles. The mixture was then placed in a water bath at 30 °C for 5-6
4 hours, with additional agitation provided every 45-60 minutes. The mixture was then removed from
5 the water bath and centrifuged at 14,000 rpm for 30 minutes. The reaction time was measured from
6 initial sonication to the beginning of centrifugation. Catalytic activity was determined from the
7 increase in free p-nitrophenol concentration, measured by UV/VIS spectroscopy at 410 nm with an
8 extinction coefficient of $8500 \text{ M}^{-1} \text{ cm}^{-1}$. The measured absorbance was compared to that of the
9 substrate solution at 410 nm and from the absorbance due to light scattering of the particle dispersion
10 of particles in 0.1 M Tris buffer (prepared identically as above) at 410 nm. These contributions to
11 the absorbance were subtracted from the total absorbance at 410 nm to obtain the absorbance due
12 to free p-nitrophenol. Each data point was calculated based on the average of 3 to 7 trials.

13 **Differentiation of catalytic activity after imprinting in non-functionalized and functionalized**
14 **silica particles**

15 Using the above procedure, the catalytic activity of functionalized and non functionalized
16 silica particles imprinted with N- α -decyl-L-phenylalanine-2-aminopyridine was measured. The
17 substrate was Suc-AAPF-PNP at a concentration of 0.2 mM. The reactions were performed in Tris
18 buffer (50 mM), pH 7.8, in a volume of 1.0 ml. Rates were converted from Absorbance (410 nm)
19 units into 11 mol units via a standard curve with p-nitroanilide. The data were normalized per mg of
20 catalyst.

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Rates are given as μmol nitroanilide/(mg catalyst min). The rate of hydrolysis using imprinted particles containing 5% amine silane, 5% carboxylate silane, and 5% imidazole silane was 5.2×10^{-5} ; the rate of hydrolysis using imprinted particles with no amine, carboxylate, or imidazole groups was 1.0×10^{-5} ; the rate of hydrolysis using non imprinted particles containing 5% amine silane, 5% carboxylate silane, and 5% imidazole silane was 3.0×10^{-6} ; the rate of hydrolysis using non imprinted particles without any functional groups was 1.9×10^{-6} . A table of these results is given below:

Table 1. Initial rate of hydrolysis of Suc-AAPF-PNP catalyzed by functionalized and non-functionalized imprinted silica particles

<u>Description</u>	<u>Initial Rate^a</u>	<u>Ratio^b</u>
5% PEDA + 5% CTES + 5% IPTS + 20% TSA	5.2×10^{-5}	27.3
No modifiers + 40% TSA	3.4×10^{-5}	17.9
No modifiers + 20% TSA	1.0×10^{-5}	5.3
5% PEDA + 5% CTES + 5% IPTS, No TSA	3.0×10^{-6}	1.6
No modifiers and no TSA	1.9×10^{-6}	1.0

a. Initial Rate in μmol nitroanilide/(mg catalyst-min)
b. Ratio of (sample)/(unmodified silica particles)

This example demonstrates that catalytic activity is greater for imprinted particles with functional groups and that non imprinted particles have far less catalytic activity.

Differentiation of catalytic activity of silica particles imprinted with different enzyme transition state analogs:

Using the above procedure, the catalytic activity of functionalized and non functionalized

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1 silica particles imprinted with N- α -decyl-L-phenylalanine-2-aminopyridine, 3-
2 octylamidophenylboronic acid and BOC-L-Phe-2-aminopyridine (a non-acylated imprint molecule
3 used as a comparative example), was measured. The substrate was DL-BAPNA, a solution of which
4 was prepared by dissolving 400-500 mg BAPNA in 5 ml dimethyl sulfoxide, and diluting 0.1 ml
5 aliquots of this preparation to 10 ml with 0.1 M Tris buffer (pH 7.4) to yield a ~0.2 mM BAPNA
6 solution. The trypsin catalyzed rate of substrate BAPNA hydrolysis has been calculated from a
7 literature report as $6.7 \mu\text{M mg}^{-1} \text{ min}^{-1}$ at 25 °C. Ester hydrolysis was performed at 30 °C. Three to
8 seven hydrolysis measurements were made for each type of particle, and an analysis of variance at
9 a 95% confidence level indicates that improvement in hydrolysis rate is provided by both the
10 presence of the functional silanes on the particle surface and the presence of the imprint molecule
11 during particle synthesis, and that there is a significant correlation between these two factors. A table
12 of the initial rate data is given below.

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Table 2. Initial rate data for the hydrolysis of DL-BAPNA catalyzed by silica particles surface-imprinted with different imprint molecules.

<u>Imprint Molecule</u>	<u>Wt. % of Added Functionalized Silanes^a</u>	<u>Initial Rate ($\mu\text{M}/\text{mg}/\text{min} \cdot 10^5$)</u>	<u>σ ($\mu\text{M}/\text{mg}/\text{min} \cdot 10$)^b</u>
None	0	0.47	0.128
None	5	0.83	0.302
3-Octylamidophenylboronic acid	0	1.04	0.26
3-Octylamidophenylboronic acid	5	1.81	0.324
BOC-L-Phe-2-aminopyridine	0	1.30	0.162
BOC-L-Phe-2-aminopyridine	5	1.41	0.156
N-Decyl-L-Phe-2-aminopyridine	0	1.37	0.302
N-Decyl-L-Phe-2-aminopyridine	5	3.98	0.489

a. Mixture of PEDA, CTES and IPTS

b. Based on 3 to 7 measurements

Particles surface-imprinted with the acylated phenylalanine and aminophenylboronic acid imprint molecules demonstrate an increased enhancement of hydrolytic activity in the presence of functional surface silanes. In contrast, particles surface-imprinted with non-acylated phenylalanine in the presence of functionalized silanes do not reveal a similar rate enhancement.

Effect of increasing amount of functional silanes and imprint molecule on hydrolytic activity.

Using the above procedure, the catalytic activity of functionalized silica particles imprinted with increasing amounts of N- α -decyl-L-phenylalanine-2-aminopyridine was measured.

The following table summarizes how the initial rate of hydrolysis of DL-BAPNA is affected by differences in the amount of functional surface silanes and amount of imprint molecule used during

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the preparation of catalytic particles:

Table 3. Initial rate data for the hydrolysis of DL-BAPNA catalyzed by silica particles surface-imprinted with N- α -decyl-L-phenylalanine-2-aminopyridine.

<u>Amount of Imprint</u> <u>Molecule (Mol%</u> <u>of Total</u> <u>Surfactant)</u>	<u>Amount of</u> <u>Functionalized</u> <u>Silanes* (Wt% of</u> <u>Total Silica)</u>	<u>Substrate</u> <u>Concentration</u> <u>(mM)</u>	<u>Initial Rate</u> <u>(μM/mg/min*10⁵)</u>	<u>σ</u> <u>(μM/mg/min*10⁵)</u>
0	5	0.2	0.83	0.30
10	5	0.2	2.65	0.42
10	5	0.4	3.41	0.33
10	10	0.4	3.92	0.89
10	15	0.4	4.17	0.38
20	5	0.2	3.98	0.49
20	5	0.4	5.55	0.15
20	10	0.4	4.12	0.37
40	5	0.2	7.11	0.97
40	5	0.4	9.13	0.32
40	15	0.4	6.28	0.62

a. Mixture of PEDA, CTES and IPTS

b. Based on 3 to 7 measurements

With three to seven hydrolysis measurements for each particle type, an analysis of variance on the results obtained in 0.4 mM substrate solution indicates that there is no significant difference in hydrolysis rate resulting from increasing the amount of functional silanes present in the particle; therefore, although the presence of a small amount of functional silanes in the catalytic particle provides some improvement in hydrolysis rate, there is no added benefit from increasing the amount

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1 of functional silanes beyond a certain level. There is, however, a significant benefit from increasing
2 the amount of imprint molecule used in the synthesis of the catalytic particles. It was found that this
3 effect is linear up to 40% added imprint molecule in the NP-5 surfactant used in the microemulsion
4 to prepare the particles.

5 **Evaluation of kinetic parameters for catalytic particles.**

6 The catalytic activity of imprinted silica particles was studied over a range of initial substrate
7 concentrations, from about 0.1 mM to 1.0 mM. Figure 1 shows data for a series of silica particles
8 synthesized with 5% incorporated functional silanes (PEDA, IPTS, CTES mixture) and 0, 10, 20,
9 and 40% added acylated phenylalanine-2-aminopyridine anilide imprint molecule. Data for
10 hydrolysis catalyzed by non-imprinted silica is included in the plot for comparison although this data
11 is not expected to follow saturation kinetics. Solid lines in the figure represent curves fitting the data
12 to the Michaelis-Menten equation,

13
$$v = \frac{K_1 S}{K_2 + S}$$

14 using parameters for K_1 and K_2 derived from non-linear regression. The following table summarizes
15 K_1 and K_m parameters for a number of different imprinted silicas:

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Table 4. Kinetic constants for the hydrolysis of DL-BAPNA catalyzed by non-imprinted silica particles and by silica particles surface-imprinted with N- α -decyl-L-phenylalanine-2-aminopyridine.

<u>Amount of</u> <u>Imprint</u> <u>Molecule</u> <u>(Mol% of</u> <u>Total</u> <u>Surfactant)</u>	<u>Amount of</u> <u>Functional-</u> <u>ized Silanes</u> <u>(Wt% of</u> <u>Total</u> <u>Silica)</u>	<u>K₁</u> <u>(μM/mg/min*10⁵)</u>	<u>σ</u> <u>(μM/mg/min*10⁵)</u>	<u>K₂</u> <u>(mM)</u>	<u>σ</u> <u>(mM)</u>	<u>K₁/K₂</u> <u>(mg⁻¹min⁻¹)</u>
10	5	7.29	0.71	0.48	0.10	0.15
11	0	5.24	0.78	0.65	0.18	0.08
12	5	9.41	0.05	0.34	0.04	0.28
13	5	12.5	0.08	0.17	0.03	0.74

a. 20 mol% (total surfactant) of imprint molecule used for imprinting.

b. Particles contain 5 wt% (total silica) of functionalized silanes (PEDA, IPTS, CTES mixture).

c. Constants calculated from non-linear regression of data in Fig. 1. Each data point was determined from at least two trials.

d. Reaction data not plotted in Fig. 1.

One observed trend is that the value of K_1/K_2 , a measure of catalytic efficiency per mg of silica, increases with increasing amount of imprint molecule used during particle synthesis, as well as when functional silanes are present in the catalyst.

Selectivity of molecularly imprinted catalysts to several substrates.

Figure 2 presents data for the hydrolysis of racemic trypsin substrate DL-BAPNA, the optically pure substrates D- and L-BAPNA, and the α -chymotrypsin substrate succinyl-Ala-Ala-Pro-Phe-p-nitroanilide. Solid lines in the Fig. 2 represent curves fitting the data to the Michaelis-Menten equation. The following table presents K_1 , K_2 and K_1/K_2 for succinyl-Ala-Ala-Pro-Phe-p-nitrophenol

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and D- and DL-BAPNA.:

Table 5. Selectivity of catalysis by silica particles surface-imprinted with N- α -decyl-L-phenylalanine-2-aminopyridine.

Substrate	K_1 ($\mu\text{M}/\text{mg}/\text{min} \cdot 10^5$)	σ ($\mu\text{M}/\text{mg}/\text{min} \cdot 10^5$)	K_2 (mM)	σ (mM)	K_1/K_2 ($\text{mg}^{-1}\text{min}^{-1}$)
Suc-AAPF-NA	2.70	0.69	0.52	0.24	0.05
DL-BAPNA	9.41	0.48	0.34	0.24	0.28
D-BAPNA	9.43	0.54	0.24	0.24	0.39
L-BAPNA	0.65	0.11	0.55	0.21	0.01

a. 20 mol% (total surfactant) of imprint molecule used for imprinting.

b. Particles contain 5 wt% (total silica) of functionalized silanes (PEDA, IPTS, CTES mixture).

c. Each data point was determined from at least two trials.

There are two interesting features of this data set. First, the catalyst particle seems to be selective for the trypsin substrate D-BAPNA over the chymotrypsin substrate, even though the imprint molecule more closely mimics the chymotrypsin substrate. Second, the particles appear to be selective for the D isomer of the trypsin substrate, even though the imprint molecule had the L-isomer configuration. In fact, the L-BAPNA is almost completely inert to the particles.

These results demonstrate that template-directed molecular imprinting is a viable method of creating robust, enantioselective catalytic silica particles. To our knowledge, this is the first example of forming catalytic silica particles by imprinting reactive sites exclusively into the surface of the particle. Our efforts represent an alternative to conventional molecular imprinting techniques. Surface-imprinting was clearly demonstrated by the effect of increasing the amount of acylated imprint molecule, N--decyl-L-phenylalanine-2-aminopyridine, on initial rate and K_1/K_2 (measure

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1 of catalytic efficiency per amount of surface-imprinted silica) values of DL-BAPNA amide
2 hydrolysis (Tables 2 and 3). As the amount of N--decyl-L-phenylalanine-2-aminopyridine used to
3 surface-imprint the silica particles increased, the catalytic efficiency of the particles increased. For
4 this to happen, the imprint molecule must act as a surfactant headgroup that is positioned at the
5 surfactant-water interface of the microemulsion to create the catalytic site. In addition, the
6 observation of enantioselectivity (Table 4) strongly suggests that defined, surface-imprinted catalytic
7 sites have been formed using template-directed molecular imprinting.

8 While both the imprint molecule and the functionalized amine and carboxylate silanes have
9 a positive effect on the hydrolysis rates obtained, it is their combination that produces the best
10 catalytic particles. The importance of the functionalized silanes can be seen from the data in Tables
11 1 and 2. The initial rates for particles imprinted in the absence of functionalized silanes indicate that
12 imprint molecule shape has only limited impact on the efficacy of the molecular recognition site
13 created. Incorporation of functionalized silanes into the particles reveals two important features of
14 this imprinting method: 1) The lack of a rate enhancement for the non-acylated chymotrypsin TSA
15 in the presence of silanes as compared to the rate enhancement observed for the acylated imprint
16 molecules strongly suggests that acylation aids in effectively positioning the imprint molecule at the
17 surfactant-water interface at which the particle forms; 2) the presence of complementary hydrogen-
18 bonding silanes at the surfactant-water interface as the silica particle is forming is essential to
19 forming reactive catalytic imprints. The results suggest that some or all of the surface amine and
20 carboxylate groups, along with the surface hydroxyl groups of the silica particles, are capable of

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1 interacting with the substrate amide carbonyl group to enhance its reactivity. The observation that
2 shape of the imprinted cavity alone does not produce effective imprinted catalytic sites within
3 polymers has been previously reported.

4 Results obtained to determine the effect of increasing the wt. % of the functionalized silanes
5 in the imprinted silica particles reveal that doing so results in a decrease in rate of amide hydrolysis
6 (Table 2). This is probably due to hydrogen bonding between the functionalized silanes leaving
7 fewer available for interaction with the substrate. From previous reports, it is known that surface
8 functional groups will form hydrogen bonds with the native surface hydroxyl groups of the silica
9 particles as well as with each other, thereby decreasing their basicity and ability to hydrogen bond
10 to other molecules.

11 The most interesting kinetic data deal with the specificity observed for amide hydrolysis
12 catalyzed by silica particles with surfaces imprinted with N--decyl-L-phenylalanine-2-
13 aminopyridine. The surface imprinted particles have selectivity for the trypsin substrate over the
14 chymotrypsin substrate even though imprinting was done with the chymotrypsin TSA (Table 4,
15 Figure 4). The magnitude of the kinetic constants obtained for the hydrolysis of L-, D-, and DL-
16 BAPNA catalyzed by the surface-imprinted silica particles are consistent with a D-enantioselective
17 mode of hydrolysis. In fact, the hydrolysis of D-BAPNA catalyzed by the surface-imprinted particles
18 is 10 times faster (K_1) and 39 times more efficient (K_1/K_2) than the hydrolysis of L-BAPNA
19 catalyzed by the particles. Based on the observed enantioselectivity, at least three of the groups
20 surrounding the chiral methine carbon of D-BAPNA must be bound to the catalytic site surface-

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imprinted silica particle. This reversal of stereoselectivity has been observed to occur for substrate hydrolysis or transformation catalyzed by enzymes such as -chymotrypsin, lipase, peptidases and -lactamases. The enantioselectivity of the hydrolysis strongly suggests that molecular structure affected substrate packing within the catalytic site. Substrate substituent effects have been observed to have a major factor influencing enzyme enantioselectivity. Since the substrate is structurally different than the imprinting molecule, the observed enantiopreference of the amide hydrolysis catalyzed by the surface-imprinted silica particles may arise because the D-BAPNA packs more readily into the imprinted catalytic site than L-BAPNA.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that the invention may be practiced otherwise than as specifically described.

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ABSTRACT

A method of making a molecularly imprinted porous structure makes use of a surfactant analog of the molecule to be imprinted that has the imprint molecule portion serving as the surfactant headgroup. The surfactant analog is allowed to self-assemble in a mixture to create at least one supramolecular structure having exposed imprint groups. The imprinted porous structure is formed by adding reactive monomers to the mixture and allowing the monomers to polymerize, with the supramolecular structure serving as a template. The resulting solid structure has a shape that is complementary to the shape of the supramolecular structure and has cavities that are the mirror image of the imprint group. Similarly, molecularly imprinted particles may be made by using the surfactant to create a water-in-oil microemulsion wherein the imprint groups are exposed to the water phase. When reactive monomers are allowed to polymerize in the water phase to form particles, the surface of the particles have cavities that are the mirror image of the imprint group.

Figure 1

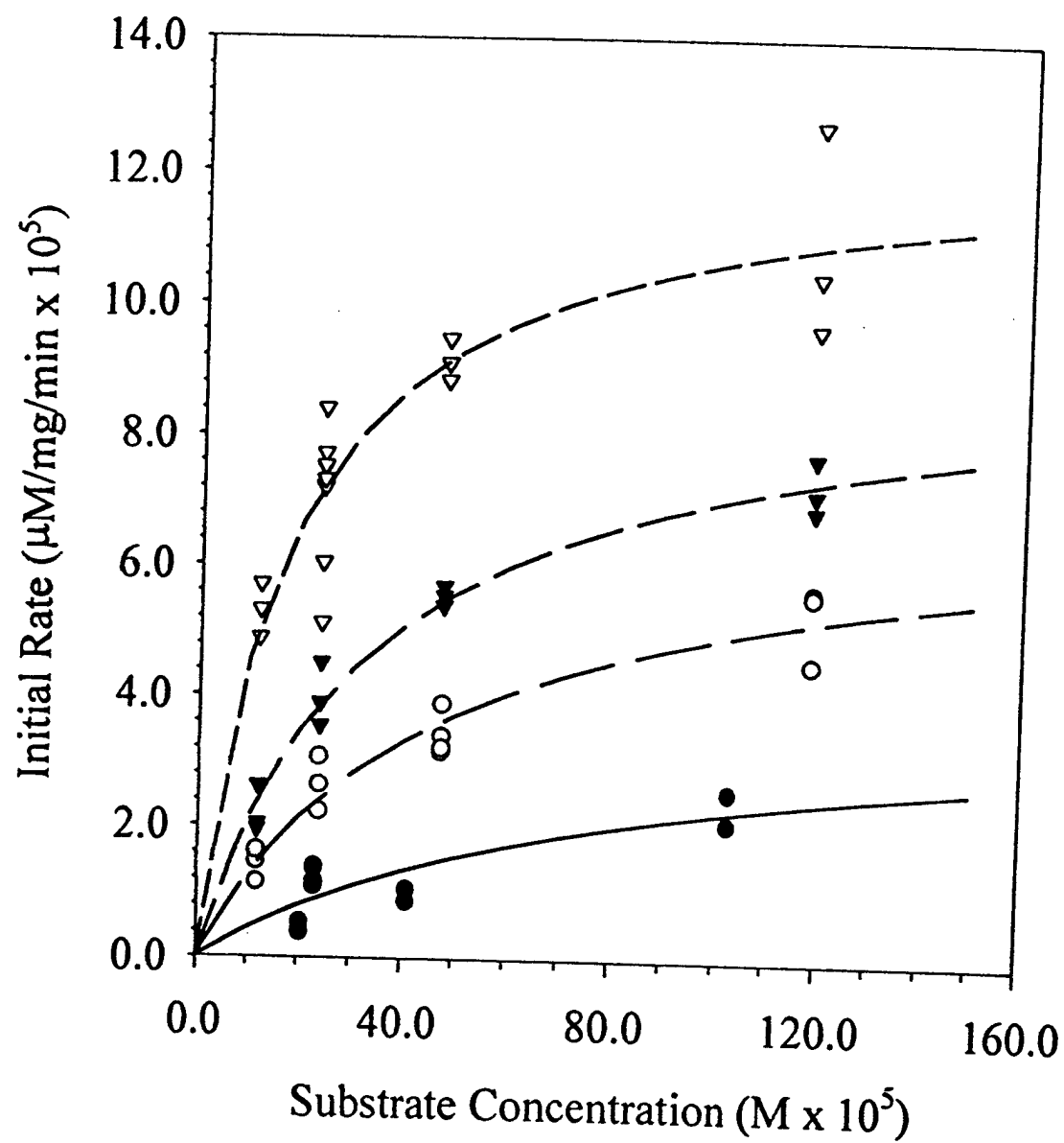


Figure 2

